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CLINICAL INVESTIGATION

Expression of CR1 (CD35) mRNA in podocytes from adult and fetal human kidneys

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Expression of CR1 (CD35) mRNA in podocytes from adult and fetal human kidneys. The presence of CR1 mRNA in podocytes was investigated using a ^{35}S-labeled CR1 cDNA probe and in situ hybridization in sections from fetal and adult human kidneys. CR1 mRNA was only detected in immature podocytes at early stages of glomerular differentiation in the fetal kidney. In contrast, CR1 antigen was abundantly expressed on immature and mature podocytes in fetal kidneys and adult glomeruli. Thus, the expression of the CR1 gene in podocytes is tightly regulated. It is possible that the prolonged life span of adult podocytes is associated with a slow turnover of CR1 and low or intermittent accumulation of CR1 mRNA transcripts.

The human complement receptor type 1 (CR1, CD35) is a polymorphic single chain membrane glycoprotein expressed on human erythrocytes, neutrophils, monocytes/macrophages, B lymphocytes, a subset of CD4+ T lymphocytes, follicular dendritic cells, Küpffer cells and human adult and fetal podocytes [1–12]. By immunoelectron microscopy, CR1 antigen has been observed on the plasma membrane, in coated pits [13] and in the Golgi apparatus [6] of adult podocytes. The number of CR1 molecules expressed in adult podocytes has been estimated to be 2.10^5 per cell [14]. Using in situ hybridization, we have now examined the presence of specific CR1 mRNA in podocytes from adult and fetal human kidneys. CR1 mRNA-containing podocytes were present in immature glomeruli from fetal kidney, whereas no CR1 mRNA-containing podocytes were detected in adult kidney sections. This is the first example, in the human kidney, of the presence of a specific mRNA in cells during ontogenesis which becomes undectectable in normal mature glomeruli, although the antigenic expression of the gene product persists.

Methods

Tissues

Normal adult kidney tissue specimens were obtained from normal parts from two kidneys immediately after surgical nephrectomy for renal cell carcinoma. Human fetal kidneys were obtained from 20 and 26 week pregnancies according to the guidelines of the French National Committee of Ethics. Tonsil tissues were obtained following elective surgery. Tissue specimens were immediately quick-frozen and stored in liquid nitrogen until sectioned.

The 0.75 kb CR1-1 cDNA probe from human tonsil [15] was from D.T. Fearon (Johns Hopkins Medical School, Baltimore, Maryland, USA). The insert was excised from the plasmid using Eco R1 (Appligene, Illkirch, France) and isolated by electrophoresis prior to labeling. For in situ hybridization, the CR1 cDNA was labeled with ^{35}S dCTP (Amersham, Les Ulis, France) using random oligonucleotides as primers to a specific activity of 0.9 to 2.10^9 cpm/μg. The labeled probe was ethanol-precipitated and resuspended at 10 μg/ml in 10 mM Tris-HCL, 1 mM EDTA, pH 7.5. Controls were a 0.9 kb fragment of the recombinant plasmid, and a renin cDNA (from Dr. P. Corvol, INSERM U36, Paris, France) labeled to the same specific activity.

In situ hybridization

In situ hybridization was performed as previously reported [16] with minor modifications. Briefly, frozen tissue sections fixed in 3.7% paraformaldehyde in phosphate buffer saline (PBS) were sequentially digested with 0.2 N HCl and 10 μg/ml proteinase K for 30 minutes at 37°C. After dehydration and air-drying, ^{35}S-labeled CR1 cDNA probe (10^6 cpm/slide) in 15 μl of hybridization mixture was applied to sections. The hybridizing mixture contained: 50% Dowex AG deionized formamide, 10% dextran, 10 mM Tris pH 7.4, 1 mM EDTA, NaCl 0.6 N, 1× Denhardt solution, 10 mM DTT, yeast tRNA (10 mg/ml), sheared salmon sperm cDNA (400 μg/ml), and herring sperm DNA (400 μg/ml). The sections were covered with siliconized glass coverslips and sealed. The slides were incubated, for 18 hours at 37°C, de-coverslipped and washed in a solution containing 50% formamide and 50% 8×SSC for 15 minutes at 20°C, twice in 2×SSC for 30 minutes at 20°C and in 2×SSC for 24 hours at 20°C under gentle agitation. Slides were dehydrated in graded alcohols before being dipped into a 50% NTB2 emulsion (Kodak) in water. Autoradiography was for two weeks (human tonsils) and eight weeks (kidneys) in the dark at 4°C. Autoradiographies were developed in Kodak D19 (1.5 to 3 minutes,

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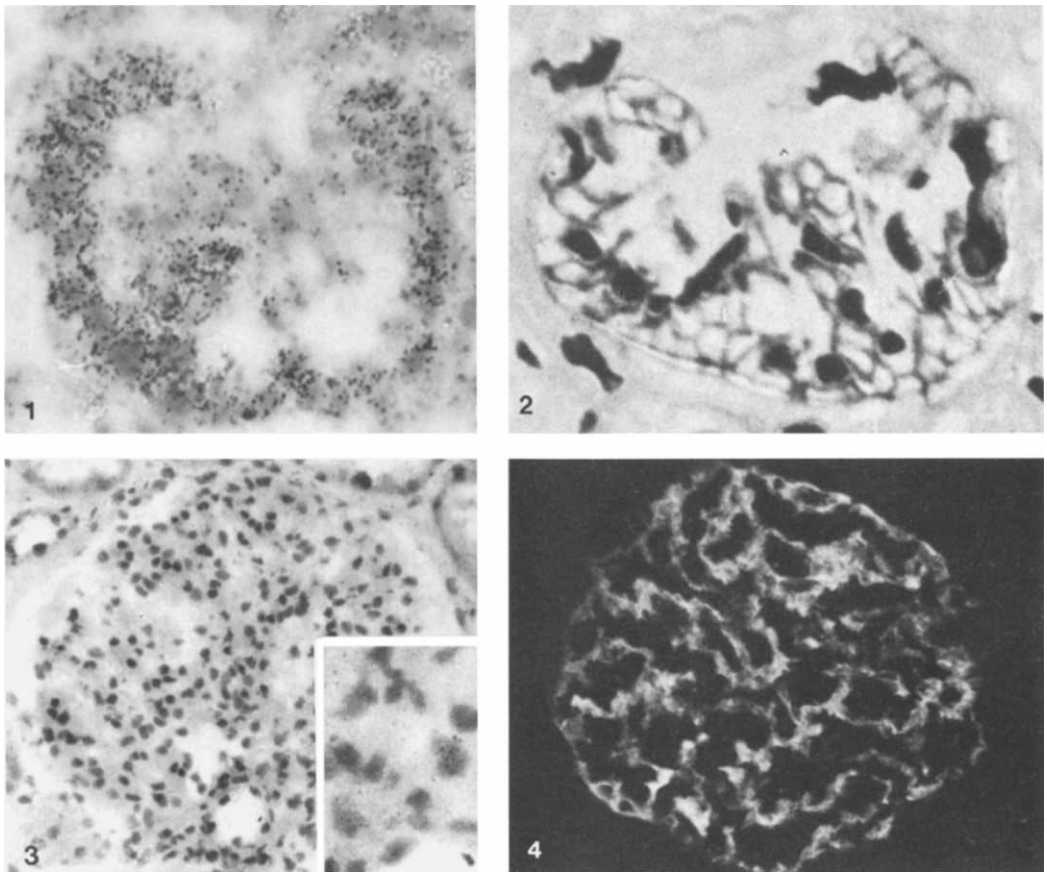

Fig. 1. *In situ hybridization with the ^{35}S CR1-1 DNA probe of a human fetal kidney section (20 gestation weeks).* Hybridizing cells are present at the periphery of an immature glomerulus at the open glomerulus maturation stage. (× 500)

Fig. 2. *Immunoperoxidase detection of CR1 antigen in a semithin section of a human fetal kidney using Fab'$_2$ rabbit anti-CR1 antibodies and peroxidase-labeled Fab'$_2$ sheep anti-rabbit antibodies.* The podocyte membrane is stained by the reaction product of peroxidase labeled second antibody (× 500). The glomerulus is at the same stage of differentiation as that shown in Fig. 1. Similar results were obtained using the monoclonal anti-CR1 antibody J3D3 [20] (Data not shown).

Fig. 3. *In situ hybridization with the CR1 cDNA CR1-1 probe of an adult human kidney section.* No mRNA-containing cell is detectable in the glomerulus. (× 250). Insert: detailed view of the same glomerulus (× 500).

Fig. 4. *Indirect immunofluorescence detection of CR1 antigen in a cryostat section of a human adult kidney using Fab'$_2$ rabbit anti-CR1 antibodies and FITC-labeled sheep anti-rabbit antibodies.* The CR1 antigen is detected in all the podocytes of the glomerulus (× 250).

20°C) and fixed in Kodak rapid fix for five minutes (20°C). As a final step, the slides were stained with hematoxylin and eosin.

Negative control experiments included 1) pretreatment with RNase A (Sigma) at a concentration of 40 μg/ml in 2×SSC for 30 minutes at 37°C prior hybridization, 2) hybridization of some slides with ^{35}S-labeled recombinant plasmid, 3) hybridization of some slides with ^{35}S-labeled renin cDNA.

A positive control experiment was performed by incubating frozen sections of human tonsils with CR1 cDNA probe [15].

Quantitation of in situ hybridization signal by image analysis

Image analysis was performed on CR1 probe-hybridized sections from two fetal kidneys and two adult kidneys, and on one RNase A-treated fetal kidney section. We used a computer vision image processor (NS 1500, Nachet-Vision, France), designed according to the "mathematical morphology" theory [17]. Silver grains segmentation was easy at the magnification ×100. Silver grains were isolated by "top hat" transformation, to select them according to their size and contrast [18, 19]. Because of the silver grain image overlapping, their surface rather than their absolute number was calculated. For silver grains localization, the operator displaced a circular mask upon immature glomerular structures, mature glomerular structures and tubular structures. The results were expressed per area unit (100 μm^2). Five measurements were performed on twenty immature glomeruli, on ten mature glomeruli from fetal kidneys and five measurements were performed on ten glomeruli of adult kidney. A Newman-Keuls test was performed in a one

way analysis of variance on measurements. Results were presented as histograms.

Immunohistochemistry

Alternate cryostat sections of specimens were air dried and acetone fixed for 10 minutes at room temperature for the detection of CR1 antigen by indirect immunofluorescence, using mouse monoclonal or polyclonal rabbit antibodies against CR1 [6, 20]. The second antibody was either goat anti-mouse fluoresceinated antibody or sheep anti-rabbit fluoresceinated antibody (Biosys, Compiegne, France). Some fetal kidney specimens were stained with Fab'_2 anti-CR1 polyclonal antibodies [6] followed by peroxidase-labeled Fab'_2 sheep anti-rabbit IgG, and processed for immunoelectron microscopy as previously described [11].

Results

Fetal and adult human kidneys

CR1 mRNA-containing cells were detected in immature glomeruli from fetal kidneys from the stage of open glomerulus to that of plurilobulated glomerulus. No hybridizing cells were detected in fully lobulated glomeruli, that is, in the most mature stage of glomeruli. In glomeruli at the open glomerulus stage, the mRNA-containing cells appeared to be ring-like, and were distributed at the periphery of the glomeruli in the area where immature podocytes were present [11] (Fig. 1). The distribution of hybridizing cells was closely related to that of CR1 antigen at that stage (Fig. 2). In glomeruli at the plurilobulated glomerulus stage, the number of mRNA-containing podocytes was lower than that of CR1-immunoreactive podocytes (data not shown). In mature fetal glomeruli and in adult kidney sections, no mRNA-containing cells were detected, although CR1 antigen was abundantly expressed on podocytes (Figs. 3 and 4).

Image analysis confirmed in a quantitative fashion that CR1 mRNA was significantly more abundant in immature glomeruli than in mature fetal glomeruli and that no CR1 mRNA was detected in mature glomeruli from fetal kidneys nor in glomeruli from adult kidneys (Fig. 5). No CR1 mRNA was present in tubular cells in fetal and adult kidneys.

Controls

No CR1 cDNA hybridization was observed in fetal kidney sections that had been pretreated with RNase.

No hybridizing cells were detected in adult and fetal tissues using the control plasmid probe or the renin cDNA probe.

Human tonsils served as a positive control for CR1 cDNA hybridization. CR1 cDNA-hybridizing cells in human tonsils were distributed in the mantle zone and in the center of the secondary follicles (Fig. 6). The distribution of CR1 cDNA-hybridizing cells correlated with that of CR1 antigen-expressing cells.

Discussion

C3b CR1 is abundantly expressed on the plasma membrane of adult human podocytes [6, 14]. CR1 antigen is an early marker of podocyte differentiation in human fetal kidneys [11]. In adult podocytes, the CR1 molecule is functional in that it can bind C3b, which is attached to surfaces, and decay-dissociate preformed alternative pathway C3 convertase complexes [7–14].

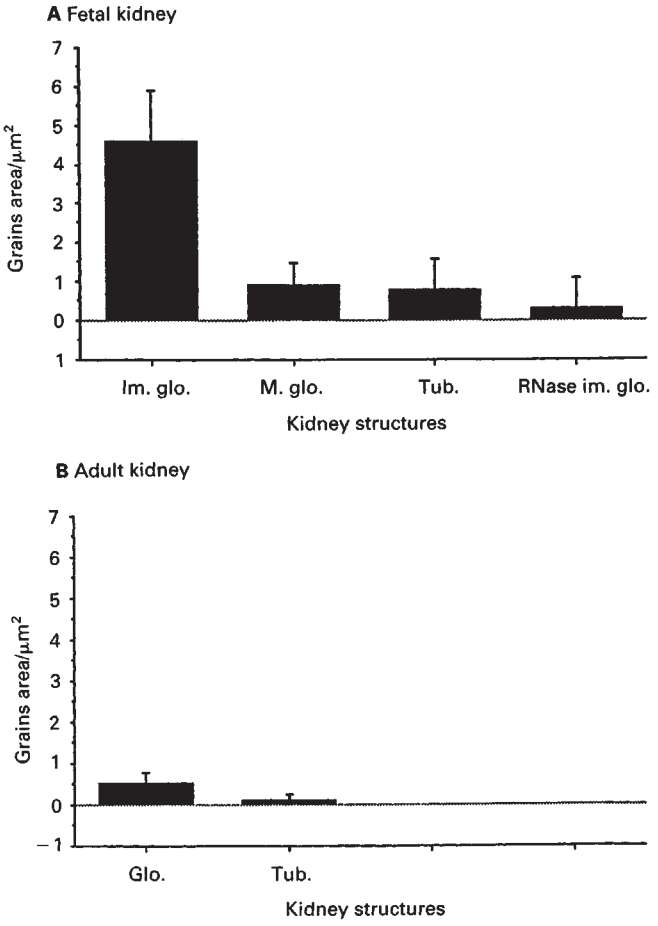

Fig. 5. *Quantitative image analysis of silver grains (grains surface per surface unit) in CR1 cDNA CR1-1-hybridized kidney sections.* The histograms depict the mean calculated surface of grains per 100 μm^2 in immature glomeruli (Im. Glo.), mature glomeruli (M. Glo.), tubules (Tub.) and in RNase-treated (control) immature glomeruli (RNase Im. Glo.) from two fetal kidneys (**A**) and in glomeruli (Glo.) and tubules (Tub.) from two adult kidneys (**B**), with 95% confidence interval.

The physiological role of CR1 on podocytes remains, however, unclear. The present study investigated the synthesis of the CR1 protein in fetal and adult human kidney by in situ hybridization.

In fetal kidneys the CR1 mRNA-containing cells with the CR1-1 cDNA probe was detected in early stages of glomerular maturation. Immunofluorescence and in situ hybridization studies indicated that CR1 is both expressed and actively synthetized by immature podocytes. CR1 mRNA expression decreased with the maturation of glomeruli. Thus, on the same cryostat section of fetal kidney, CR1 mRNA was present in immature glomeruli at the open and plurilobulated stages, and was not detected in mature (fully lobulated) glomeruli.

In adult kidney, the CR1 antigen was abundantly present on the plasma membrane of podocytes [6, 14]. However, no CR1 mRNA was detected in the cells. The latter observation is consistent with the lack of detectable CR1 mRNA in mature glomeruli in fetal kidneys. The absence of CR1 mRNA in podocytes of adult kidneys was unexpected in the light of the previous finding of CR1 antigen in the endoplasmic reticulum

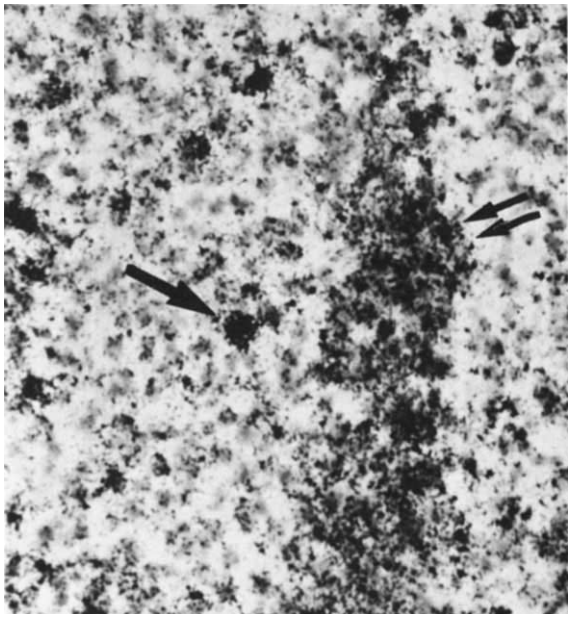

Fig. 6. *In situ hybridization with the ^{35}S CR1-1 DNA probe of a human tonsil.* Hybridizing cells are present in the germinal center (arrow) and at the periphery of the germinal center (double arrow) (× 280).

and endocytic vesicles of adult podocytes [6, 13]. It is possible that CR1 mRNA is present in adult podocytes in amounts that are below the detection threshold of in situ hybridization techniques. A long exposure of two months was required in order to detect CR1 mRNA in fetal kidney sections, suggesting that detectable CR1 mRNA levels in the kidney are low, in contrast with human tonsils in which a ten day exposure time was sufficient for CR1 mRNA detection. It is also possible that the turnover of the CR1 mRNA in adult glomeruli occurs intermittently, or alternatively, that it is very slow and that only few CR1 mRNA transcripts are sufficient to ensure the podocyte requirement for new CR1 glycoprotein molecules. Consistent with the latter hypothesis is the observation of a prolonged life span of podocytes in normal rats [21]. Newly synthetized CR1 glycoprotein would accumulate in the Golgi apparatus and may be detected by immunoelectron microscopy. The mere detection of CR1 antigen on the plasma membrane in the Golgi apparatus and in endocytic vesicles is not indicative of ongoing synthesis of the protein, since it could also reflect the intracellular traffic of the protein between the plasma membrane and the Golgi apparatus [22]. In recent studies, it was observed that specific mRNA for human elastase was detected in cells at specific differentiation stages in the myelocytic lineage. mRNA transcripts were absent in mature cells of the neutrophil myeloid series although the cells contained the protein [23].

Results of the present investigation indicate that the expression of the CR1 gene in podocyte is tightly controlled and differently regulated at various stages of cell differentiation.

We are currently examining whether CR1 mRNA may be expressed in glomeruli of diseased kidneys.

Acknowledgments

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